A METHOD FOR COLLECTING DRIED PLANT SPECIMENS FOR DNA AND ISOZYME ANALYSES, AND THE RESULTS OF A FIELD TEST IN XINJIANG, CHINA

Dried and pressed plant specimens have been used for scientific study since the Italian Renaissance (Stafleu, 1971), and this remains the most common method of preserving the "information content" of living plants. Today, the standard herbarium specimen is routinely augmented by the preservation of material for anatomical, cytological, or chemical analysis (Davis & Heywood, 1963).

The growing application of molecular data in plant systematics requires methods for preserving material from which isozymes and nucleic acids can be recovered. In most cases, it has been considered necessary to transport plants quickly to the laboratory on ice, or to freeze and store plant tissues in liquid nitrogen until they can be returned to the laboratory. Both methods can be used quite successfully when the plants studied are nearby and in easily accessible localities. They have also been applied to collect material for molecular analysis in more remote regions (Sytsma & Schaal, 1985; P. Peterson, pers. comm.), but in these cases the logistics and the expense are prohibitive. In addition, the use of ice or liquid nitrogen storage is impractical when making large-scale "floristic" collections. An alternative to these methods seems most desirable.

The recovery of high molecular weight DNA from dried plant material has been demonstrated by Rogers & Bendich (1985) and Doyle & Dickson (1987). Pyle & Adams (1989) recently compared 27 treatments of plant specimens and found that only fresh, frozen, or dried plant tissues provided good yields of quality genomic DNA. They obtained high molecular weight DNA from spinach leaves following desiccation and storage in a desiccator for two months, but degradation was observed when tested at five months. These results prompted additional tests using both fresh and air-dried spinach placed directly into silica gel in jars. The initial results of this experiment are reported here.

The primary purpose of this report is to describe a field test of a simple and inexpensive drying method for preserving plant material for subsequent nucleic acid and isozyme recovery. This method is appropriate for use in remote regions and can easily complement the routine collection of herbarium specimens.

Materials and Methods

Preparation of plant material, DNA extraction, and gel electrophoresis of genomic DNA from fresh spinach (*Spinacia oleracea* L.) followed the protocol of Pyle & Adams (1989) with the following modification: Fresh and air-dried (42°C, 36 hr., in a plant press) leaves were placed directly in contact with silica gel, sealed in air-tight plastic bottles, and stored at 37°C for five months.

A field test was conducted during a 24-day expedition in the Xinjiang Uygur Autonomous Region, People's Republic of China. This remote region of northwestern China is characterized by a strongly continental climate. Collections were made in the Tarim Basin, Songarian Basin, and Tien Shan Mountains from sea level to 3,800 m. Approximately 2–5 grams of plant tissue were wrapped in tissue paper (to prevent plant tissue fragmentation) and placed in a 125-ml Nalgene bottle prefilled to one-third capacity with anhydrous $CaSO_4$, or Drierite (W. A. Hammond Co., Xenia, Ohio). Indicator Drierite was mixed with nonindicator Drierite in a 1 : 5 proportion. Bottles were labeled, tightly sealed, and stored at ambient temperature. Most plant tissue was completely dry within 24 hours. In succulent material a single change of Drierite was required.

Upon return to the laboratory, bottles were stored at −20°C. The hot CTAB procedure (Doyle & Dickson, 1987; Doyle & Doyle, 1987) was used to extract high molecular weight DNA. Best results were obtained when the dried plant material was ground in the presence of liquid nitrogen. Precipitation of DNA with isopropanol was carried out at −20°C overnight in order to increase yields. Restriction digests were carried out with the endonuclease *Hind* III for two hours at 37°C. Agarose-

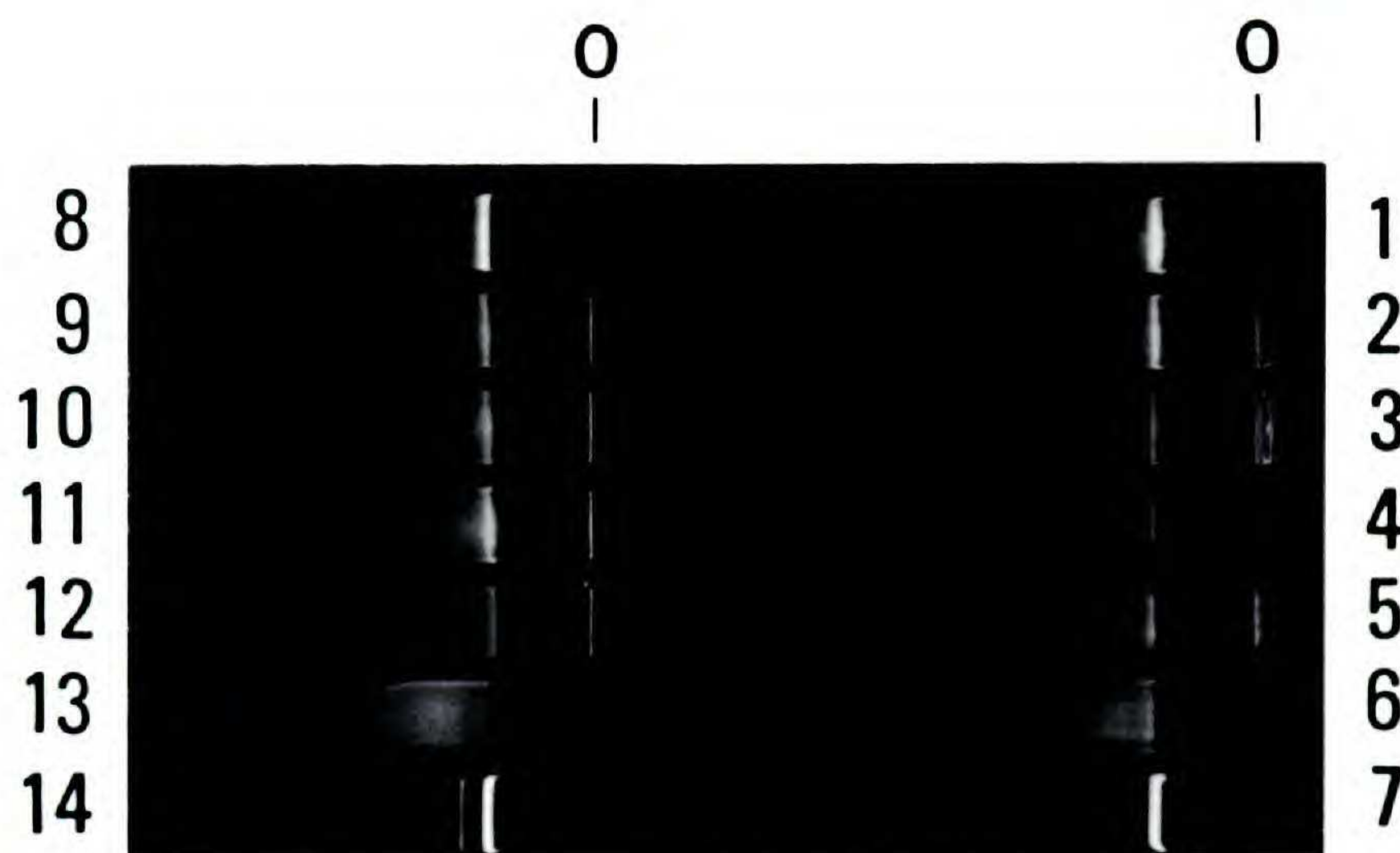

FIGURE 1. Comparison of spinach DNA from air-dried (lanes 2–6 for months 1–5) and fresh leaves stored on silica gel (lanes 9–13 for months 1–5). Lanes 1 and 8 are DNA from fresh spinach (control). Lanes 7 and 14 are lambda *Hind* III DNA markers. Size standards from the origin (O) are 23.1, 9.4, and 6.6 kb. The dual-loaded gel was run on agarose, 0.6%, 10 V/cm, 30 minutes.

gel electrophoresis and transfer of DNA from agarose gels to BioTrace (Gelman) nylon filters were conducted following standard protocols (Palmer, 1986). Preparation of digoxigenin-labeled probes, filter hybridizations, filter decolorization, and probe removal followed the manufacturer's instructions (Boehringer Mannheim Biochemicals; BMB) as modified by Rieseberg et al. (1990).

Chloroplast and nuclear ribosomal DNA variation was detected by hybridizing the filter-bound DNAs to plasmids containing a 9.9 kb *Sac* I fragment of the *Lactuca* chloroplast (courtesy of Rob-

TABLE 1. Taxa used for DNA extraction and isozyme analysis. Voucher specimens will be deposited in PEK and RSA. Collections are by Aaron Liston (L.) or James Morefield (M.). Specimens were used for successful DNA extraction (D), unsuccessful DNA extraction (X), isozyme analysis for ME, PGI, and TPI (I), and isozyme analysis for all enzymes used in unpublished studies of these genera (A).

Collection	Use	Family	Species
M. 5157	D	Asteraceae	*Artemesia dracunculus* L.
L. 827-12	I	Asteraceae	*Senecio krascheninnikovii* Schischk.
L. 829-5	D	Brassicaceae	*Lepidium latifolium* L.
M. 4993	I	Brassicaceae	*Lepidium latifolium* L.
M. 5042	D	Caryophyllaceae	*Cerastium beeringianum* Cham. & Schlecht.
L. 809-4	D I	Chenopodiaceae	*Ceratoides latens* (Gmel.) Holmgren & Reveal
M. 5040	D	Crassulaceae	*Rhodiola coccinea* (Royle) A. Bor.
L. 819-7	D	Cyperaceae	*Carex* [sect. *Lamprochlaenae* (Drejer) Bailey] sp.
L. 816-1	D A	Fabaceae	*Astragalus contortuplicatus* L.
L. 811-1	D I	Fabaceae	*Glycyrhiza inflata* Batal.
L. 813-1	I	Fabaceae	*Sphaerophysa salsula* (Pall.) DC.
L. 819-3	D	Gentianaceae	*Gentiana aquatica* L.
L. 835-22	D A	Paeoniaceae	*Paeonia anomala* L.
L. 823-8	D	Plantaginaceae	*Plantago minuta* Pall.
L. 808-9	D I	Poaceae	*Aeluropus littoralis* (Willd.) Parl.
M. 4812	D	Primulaceae	*Glaux maritima* L.
L. 819-1	I	Ranunculaceae	*Thalictrum alpinum* L.
L. 818-24	D	Rosaceae	*Potentilla fruticosa* L.
M. 5067	I	Rosaceae	*Potentilla* aff. *pennsylvanica* Ledeb.
L. 821-2	X I	Rosaceae	*Rosa platyacantha* Schrenk
L. 821-3	I	Rosaceae	*Rosa* sp.
L. 822-1	I	Rosaceae	*Rosa platyacantha* × *Rosa* sp.
M. 5200	D	Scrophulariaceae	*Lagotis integrifolia* (Willd.) Schischk.

ert Jansen) and a single 18S–25S rDNA repeat from *Helianthus argophyllus* Torr. & Gray (courtesy of Mike Arnold).

Sample preparation and enzyme electrophoresis followed the general methodology of Soltis et al. (1983). The tris-HCl grinding buffer-PVP solution described by Soltis et al. (1983) was used for enzyme extraction from dried plant tissue. All enzymes were resolved on 12% starch gels. The glycolytic enzymes malic enzyme (ME), phosphoglucoisomerase (PGI), and triosephosphate isomerase (TPI) were examined. A modification of gel and electrode buffer system 8 (Rieseberg & Soltis, 1987) was used to resolve these enzymes.

Results

Figure 1 shows genomic DNA from air-dried (lanes 2–6 for months 1–5) and fresh spinach (lanes 9–13 for months 1–5). Lanes 1 and 8 are DNA from fresh spinach (control). Lanes 7 and 14 are Lambda *Hind* III markers. Air-dried spinach DNA shows little change after four months storage at 37°C. However, the fresh spinach, placed directly in silica gel, sealed and stored at 37°C, always appears to be a little more degraded than air-dried and desiccated spinach. Both treatments appear to be degraded after five months storage.

Genomic DNA isolated from 15 species, representing 14 families, collected in China (Table 1) shows little or no degradation after four to six weeks of storage at ambient temperature (Fig. 2). Genomic DNA could not be successfully isolated from a species of the genus *Rosa* (Table 1). Figure 3 shows the results of hybridization of these same DNAs to the *Helianthus* 18S–25S rDNA repeat. Successful results were also obtained upon hybridization to the 9.9 kb *SacI Lactuca* chloroplast DNA probe (not shown).

Activity for the glycolytic enzymes PGI and TPI was obtained for 12 species representing eight families (Fig. 4). However, only two of these species (*Sphaerophysa salsula* and *Thalictrum alpinum*) had discernable activity for ME.

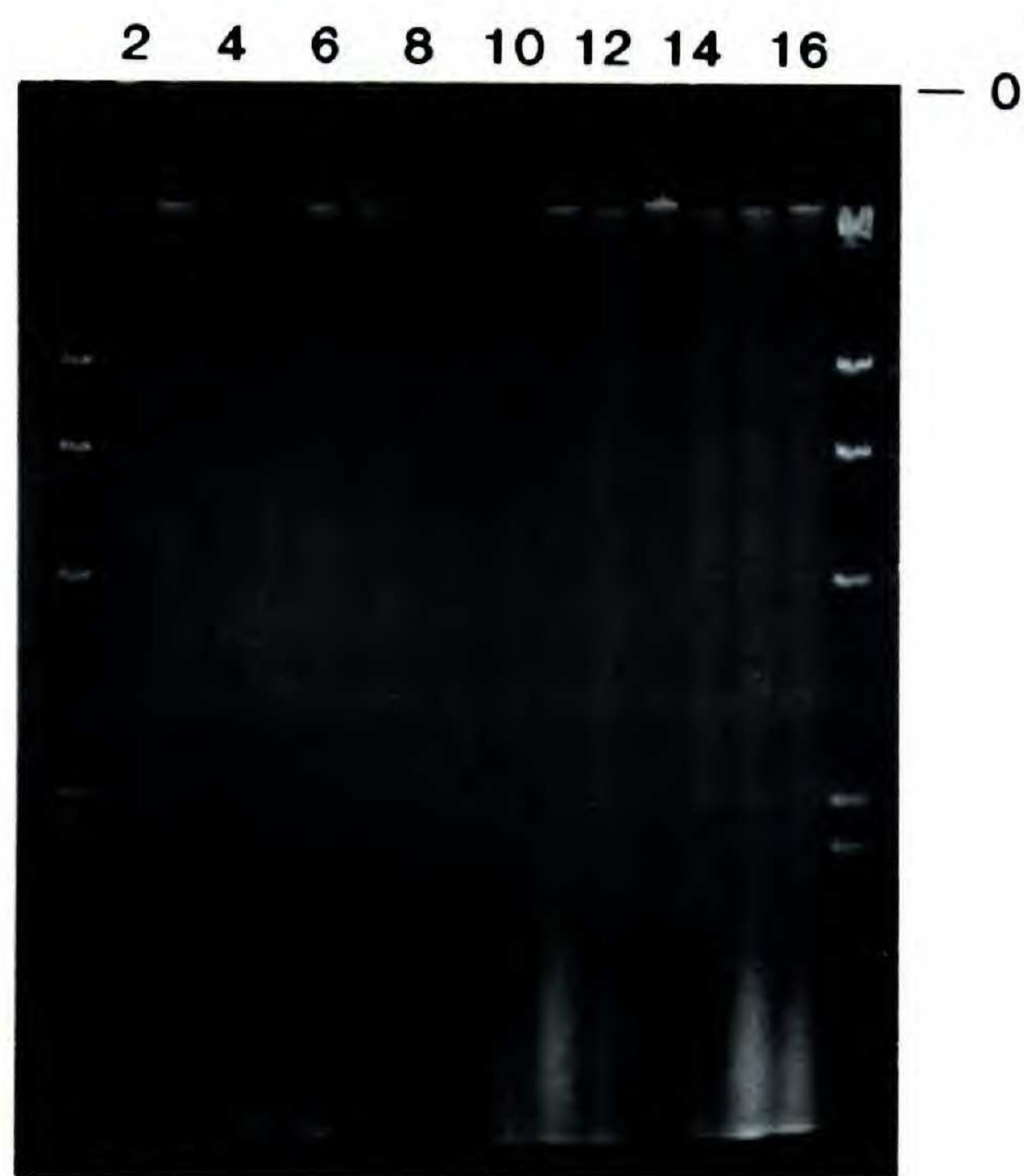

FIGURE 2. Undigested DNA from plants collected in Xinjiang, China, according to the described protocol. Lanes 1 and 17 are lambda *Hind* III DNA markers. Size standards from the origin (O) are 23.1, 9.4, 6.6, 4.4, 2.3, and 2.0 kb. The gel was run on agarose, 0.7%, 5 V/cm, 12 hours.—Lane 2. *Aeluropus littoralis.*—Lane 3. *Artemesia dracunculus.*—Lane 4. *Astragalus contortuplicatus.*—Lane 5. *Carex* sp.—Lane 6. *Cerastium beeringianum.*—Lane 7. *Ceratoides latens.*—Lane 8. *Gentiana aquatica.*—Lane 9. *Glaux maritima.*—Lane 10. *Glycyrhiza inflata.*—Lane 11. *Lagotis integrifolia.*—Lane 12. *Lepidium latifolium.*—Lane 13. *Paeonia anomala.*—Lane 14. *Plantago minuta.*—Lane 15. *Potentilla fruticosa.*—Lane 16. *Rhodiola coccinea.*

Discussion

The preservation of plant samples for DNA and isozyme analysis using only a drying agent and plastic bottles is simple, inexpensive, and of wide potential application. However, a few additional factors should be considered before the method is used in a particular study:

(1) Although genomic DNA was successfully isolated from 15 of 16 species examined, difficulty was encountered with the genus *Rosa*. Difficulties were initially found with material of the genus *Brongniartia* (Fabaceae) similarly collected in Mexico (O. Dorado, pers. comm.). It should be noted that DNA isolation from fresh material of these genera is also problematic due to the presence of polysaccharides, and alternative extraction procedures may be necessary.

(2) The examined species represented diverse growth forms including annuals, herbaceous perennials, succulents, and small shrubs. No trees were included in our study, but preliminary data from the genus *Arbutus* (Ericaceae) suggests that our technique can be used with woody taxa as well (Liston, unpublished). This technique has also been successfully used in recent collections of Ecuadoran Bromeliaceae (G. Brown, pers. comm.).

(3) Only three glycolytic enzymes were examined in the present study and no attempts were made to optimize the detection conditions for each species. In a preliminary analysis of 15 enzymes in the genera *Astragalus* and *Paeonia*, activity compa-

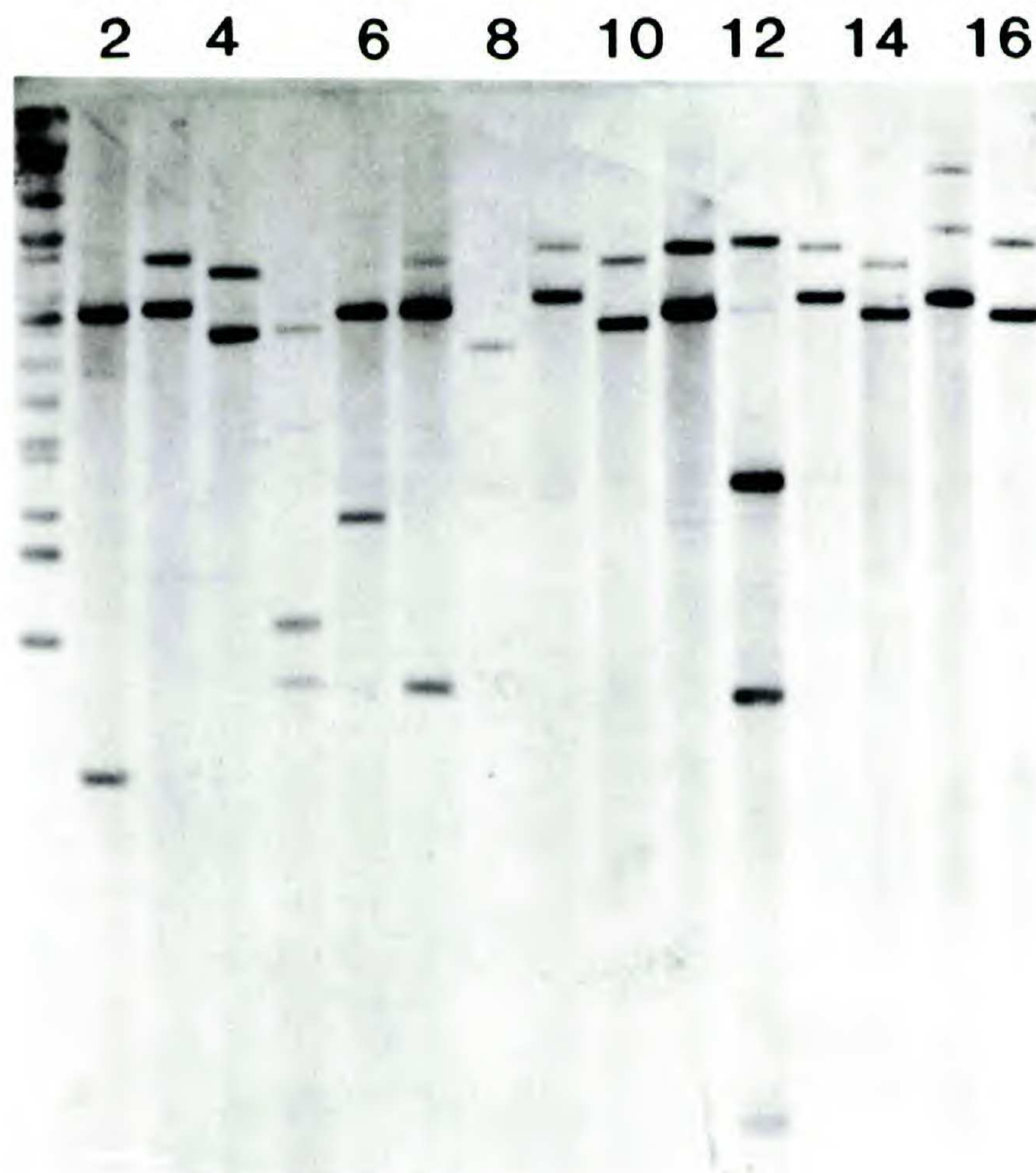

FIGURE 3. Southern blot of DNA from plants collected in Xinjiang, China, digested with *Hind* III and hybridized to a single 18S-25S rDNA repeat from *Helianthus argophyllus*. Lane 1 is lambda *EcoR* I, *Hind* III, *Sal* I, and *Sma* I DNA markers. Size standards (origin not shown) from the top of the gel are 32.7, 23.1, 21.2, 19.4, 15.3, 12.2, 9.4, 8.6, 8.3, 7.4, 6.7, 5.8, 5.6, 4.9, 4.4, and 3.5 kb. Lanes 2-16 are as in Figure 2.

rable to that of fresh material has been found in dried material of the Chinese species *A. contortuplicatus* and *P. anomala* (Liston & Zona, unpublished). Further study is needed to demonstrate the usefulness of this technique in actual isozyme studies.

In conclusion, we suggest that researchers interested in applying this technique to a particular taxon test the material beforehand, although our high rate of successful DNA isolation from a wide range of taxa suggests that it is worthwhile for any collector to obtain samples in this manner in ad-

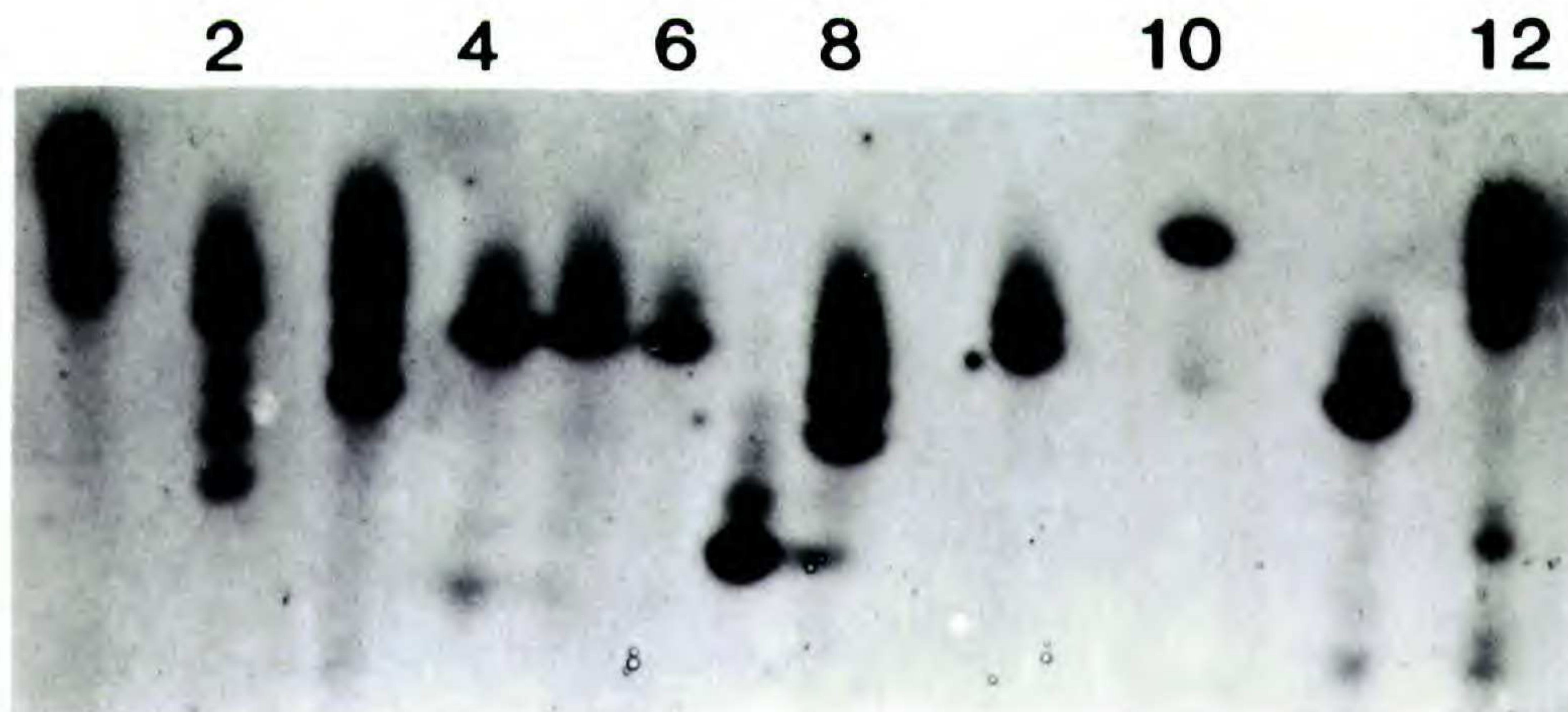

FIGURE 4. Enzyme activity for TPI from plants collected in Xinjiang, China, according to the described protocol.—Lane 1. *Aeluropus littoralis*.—Lane 2. *Ceratoides latens*.—Lane 3. *Glycyrhiza inflata*.—Lane 4. *Rosa platyacantha*.—Lane 5. *Rosa* sp.—Lane 6. *Rosa platyacantha* × *Rosa* sp.—Lane 7. *Paeonia anomala*.—Lane 8. *Sphaerophysa salsula*.—Lane 9. *Thalictrum alpinum*.—Lane 10. *Senecio krascheninnikovii*.—Lane 11. *Potentilla* aff. *pennsylvanica*.—Lane 12. *Lepidium latifolium*.

dition to herbarium specimens. We also recommend that DNA extraction and isozyme analysis be carried out as soon as possible after reaching an appropriate laboratory, because studies with spinach indicate the possibility of degradation after several months of storage. Alternatively, samples can be frozen for long-term storage.

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